



DECLARATION

I, Ai FUJII, of HIRAKI & ASSOCIATES, do solemnly and sincerely declare as follows:

1. That I am well acquainted with the English and Japanese languages and am competent to translate from Japanese into English.
2. That I have executed, with the best of my ability, a true and correct translation into English of Japanese Patent Application No. 189137/2002 filed on June 28, 2002, a copy of which I attach herewith.

This 14th day of June, 2006



Ai FUJII



[NAME OF DOCUMENT] DESCRIPTION

[Title of the Invention] ANTI-CHICKEN COCCIDIOSIS COMPOSITION

[Scope of the Claim]

[Claim 1] An anti-chicken coccidiosis composition for oral administration comprising an antibody obtained from an egg of a chicken immunized with an antigenic outer membrane protein or an immunogenic fragment thereof having a common immunogenicity shared among sporozoite and merozoite of *Eimeria acervulina*, *Eimeria tenella* and *Eimeria maxima* which are associated with chicken coccidiosis.

[Claim 2] A composition according to claim 1 further comprising a lactic acid bacterium.

[Claim 3] A composition according to claim 1 or 2 further comprising an antibody obtained from an egg of a chicken immunized with *Clostridium perfringens*.

[Claim 4] A composition according to any one of claims 1 to 3 which is used for prevention or treatment of chicken coccidiosis.

[Claim 5] An avian feed comprising the composition according to any one of claims 1 to 4.

[Claim 6] A method for preventing or treating chicken coccidiosis wherein the antibody of claim 1 is orally administered to a bird optionally in combination with a lactic acid bacterium and/or an antibody obtained from an egg of a chicken immunized with *Clostridium perfringens*.

[Detailed Description of the Invention]

[0001]

[Technical Field to Which the Invention Pertains]

This invention relates to a composition which is effective in preventing and treating chicken coccidiosis. To be more

specific, this invention relates to a composition which has excellent prophylactic and therapeutic effects for coccidiosis of chickens and other birds, which is effective in preventing the infection of chicken coccidiosis, which is effective in improving clinical conditions such as body weight loss associated with the chicken coccidiosis, or which is highly effective in treating the chicken coccidiosis.

[0002]

[Prior Art]

Chicken coccidiosis (coccidiosis in chickens) is caused by infection with a protozoan of genus *Eimeria*. In the case of chickens, symptoms and habitat of the parasite differ depending on the species of the *Eimeria*. The *Eimeria* species that are pathogenically important are the following 5 species, namely, *Eimeria tenella*, *E. necatrix*, *E. maxima*, *E. brunetti*, and *E. acervulina*, and other known *Eimeria* species include *E. mitis*, *E. praecox*, *E. hagani*, and *E. mivati*. In the case of quails, the coccidiosis is known to be caused by *E. uzura*, *E. tsunodai*, and the like; in the case of turkey, *E. meleagrimitis*, *E. adenoides*, and *E. gallopovonis* are known to be responsible; and in the case of guinea fowl, *E. grenieri* and *E. numidae* are known to be responsible. The coccidiosis is one of the chicken diseases that make most devastating damage, and the infection is established by oral intake of the oocyst. The symptoms include various types of diarrhea, anorexia, asitia, and weight loss, and the infected bird often dies. The birds are infected irrespective of their age (by day), sex, or variety. Although the coccidiosis is more frequently found in free range chicken coop, outbreak of coccidiosis has been recently reported also from egg-laying hens of multi-layered cages.

It is also to be noted that the symptoms of coccidiosis are more serious when the bird is simultaneously infected with *Clostridium perfringens*. Prevention of coccidiosis is generally accomplished by preventing cage contamination by oocysts, and by the use of anticoccidial drugs. Various synthetic anticoccidial drugs for birds and ionophorous antibiotics are used as preventive anticoccidial drugs, and a sulfa drug or a mixture of a sulfa drug and a pyrimidine drug are used for the treatment. Vaccines are also used on commercial bases.

[0003]

[Problems to Be Solved by the Invention]

In the case of chickens, the anticoccidial drugs can be used only within 10 weeks after hatchling, and in the case of a broiler chickens, it is mandatory to stop the drug administration at least 7 days before the shipping because eggs and meats are allowed to be shipped only after certain period has passed after the last administration of the anticoccidial drugs. It is this period during which use of the anticoccidial drugs are prohibited that outbreak of chicken coccidiosis is likely to develop. Another problem is increase of drug resistant chicken coccidium, and each farm, therefore, needs to select anticoccidial drugs that are effective for their farm. There was a time when a quite large number of anticoccidial drugs for chicken coccidiosis were developed. However, use of the anticoccidial drugs have been recently restricted in consideration of the action of the residual anticoccidial drugs, adverse effects on human and other animals, emergence of drug resistant chicken coccidium, and the like. In the case of Europe, use of anticoccidial drugs will be banned by the year 2007. Use of anticoccidial drugs has also been strictly

prohibited in Japan because of the enforcement of the regulatory law on feed additives.

[0004]

Concerning live vaccines for oral administration, attempts have been made to systematically immunize chickens with the strain of weak toxicity, thereby preventing infection by a highly toxic strain. An example of such live vaccine is the one comprising a mixture of oocysts from several species, which is administered to chicks so that the chicks will be infected to a mild non-fatal degree and acquire immunity. However, there is a nonnegligible downside in this type of avian coccidium live vaccine that the chicken farm is left contaminated. On the other hand, inactivated vaccines produce only insufficient amount of antibody for preventing the infection, and are not sufficiently effective. It is also to be noted that, the vaccine needs a period of several weeks before production of the protective antibody even if chicks were immunized, and this period is far too long for use in broiler chicks.

[0005]

When a chicken is suffering from chicken coccidiosis, enteric flora of the chicken becomes disturbed and symptoms like diarrhea are induced. This diarrhea continues for a particularly long period to cause physical exhaustion as well as deterioration of immunity. The birds are then subject to suffer from necrotizing enterocolitis.

[0006]

Japanese Patent Nos. 2548115, 2698778, and 2698779 disclose passive immunization of mammals using an avian antibody. This passive immunization is used in the course when a xenoantibody

(from chicken egg) is administered for the purpose of passive immunization (and in particular, by injection or other parenteral administration), and in this process, the animals are preliminarily given a food containing an antibody so that oral immunotolerance is established before the prophylactic or therapeutic administration of an antibody, and thereby preventing anaphylactic shock and serum sickness. Accordingly, this process is absolutely different from the idea of the present invention wherein protection of the animal is sought by oral passive immunization. The inventors of the present invention also filed a patent application (Japanese Patent No. 2034005) directed to an oral prophylactic or therapeutic agent for colibacillosis of preweaning pig or cow, which is produced by immunizing a chicken with at least one antigen selected from 987P, K88, and K99 antigens of enterotoxigenic E. coli which is responsible for porcine colibacillosis and K99 antigen of enterotoxigenic E. coli which is responsible for bovine colibacillosis, and recovering the antibody from the egg produced by the immunized chicken; and this agent contains a polyclonal antibody specific for such antigen. The inventors of the present invention have also filed a patent application (Japanese Patent No. 2615673) directed to a material for suppressing a food poisoning bacterium in edible fowl, which comprises whole egg, egg yolk, or antibody-containing fraction of the egg produced by a chicken which has established an immunity by preliminarily inoculation of the food poisoning bacterium, and which contains an antibody specific for the food poisoning bacterium. While these patents may disclose usefulness of the chicken egg antibody, they only disclose protection of an animal against a bacterium by oral passive immunization, and not the

protection against a protozoan. To this day, the idea of using passive immunization for protozoan remains unknown, while the idea of using the chicken egg antibody for orally inducing passive immunization against bacteria or virus may have been known to the art.

[0007]

It is also to be noted only few articles report passive immunization of birds against chicken coccidium while many articles disclose vaccines using various antigens against chicken coccidium. There is an article wherein a murine monoclonal antibody against gametocytes of *Eimeria maxima* is produced to confirm passive immunization against chicken coccidiosis (Infection and Immunity, 56: 972-976, 1988, Infection and Immunity, 58: 557-562, 1990). In this article, however, the test is conducted by intravenous injection of the murine monoclonal antibody, and not by oral administration, and accordingly, this article is far from being practical.

[0008]

In view of the situation as described above, the inventors of the present invention has made an intensive study, and found that, when a chicken, quail, or other bird is immunized with an antigenic outer membrane protein or an immunogenic fragment thereof as an antigen which has an immunogenicity commonly shared among the sporozoite and the merozoite of *Eimeria acervulina*, *Eimeria tenella*, and *Eimeria maxima* associated with coccidiosis in chickens in order to produce an anti-chicken coccidiosis antibody, then the antibody in the egg produced by such bird is administered to a chicken or other bird, the antibody attaches to the sporozoite or the merozoite of the protozoan of the *Eimeria* species to inhibit

attachment and invasion of the sporozoit or the merozoite to epithelial cell, thereby destroying pathogenicity of the sporozoit and the merozoite. The inventors of the present invention have also found that, when the anti-chicken coccidiosis antibody of the present invention is administered in combination with a lactic acid bacterium, excellent prophylactic and therapeutic effects for chicken coccidiosis is achieved through improvement of enteric flora and enhancement of immunity, and through more efficient action of the antibody of the present invention. The inventors also found that, when the anti-chicken coccidiosis antibody of the present invention is administered in combination with an antibody obtained from an egg of a chicken immunized with *Clostridium perfringens*, excellent prophylactic and therapeutic effects for chicken coccidiosis is attained through prevention of the worsening of the symptoms according to mixed infection by the *Clostridium perfringens*, and through more efficient action of the antibody of the present invention. The present invention has been completed on such findings.

[0009]

[Means to Solve the Problems]

This invention includes the inventions as described below.

(1) An anti-chicken coccidiosis composition for oral administration comprising an antibody obtained from an egg of a chicken immunized with an antigenic outermembrane protein or an immunogenic fragment thereof having a common immunogenicity shared among sporozoit and merozoite of *Eimeria acervulina*, *Eimeria tenella* and *Eimeria maxima* which are associated with chicken coccidiosis.

(2) A composition according to the above (1) further comprising a lactic acid bacterium.

(3) A composition according to the above (1) or (2) further comprising an antibody obtained from an egg of a chicken immunized with *Clostridium perfringens*.

(4) A composition according to any one of the above (1) to (3) which is used for prevention or treatment of chicken coccidiosis.

(5) An avian feed comprising the composition of any one of the above (1) to (4).

(6) A method for preventing or treating chicken coccidiosis wherein the antibody of the above (1) is orally administered to a bird optionally in combination with a lactic acid bacterium and /or an antibody obtained from an egg of a chicken immunized with *Clostridium perfringens*.

[0010]

[Mode for Carrying out the Invention]

The present invention is described in further detail.

[0011]

The antigen used in the present invention is an antigenic outermembrane protein or an immunogenic fragment thereof which has an immunogenicity commonly shared among sporozoite and merozoite of *Eimeria acervulina*, *Eimeria tenella*, and *Eimeria maxima* which are associated with chicken coccidiosis.

[0012]

Since this antigen has an immunogenicity commonly found in sporozoite and merozoite of *Eimeria acervulina*, *Eimeria tenella*, and *Eimeria maxima*, the product produced by using this antigen is

effective irrespective of whether the pathogen is *Eimeria acervulina*, *Eimeria tenella*, or *Eimeria maxima*.

[0013]

Examples of such antigens which may be used include the soluble protein (F3) or 3-1E protein with the size of 18 to 27 kilodaltons described in *Avian Diseases*, 44: 379-389, 2000; the merozoite protein with the size of 21 kilodaltons described in *J. Parasitol.*, 84: 654-656, 1998; and the protective fraction protein (FV) described in *Infection and Immunity*, 59: 1271-1277, 1991.

[0014]

The "immunogenic fragment" is not particularly limited as long as it includes at least one epitope and it can be used as an immunogen.

[0015]

Since there has been reported that an antibody could recognize an amino acid sequence comprising three amino acid residues (F. Hudecz et al., *J. Immunol. Methods*, 147: 201-210, 1992), a peptide comprising three or more amino acid residues can be postulated as the minimum unit of the immunogenic fragment. The immunogenic fragment, however, is a peptide or a polypeptide preferably comprising five or more amino acid residues, and more preferably comprising ten or more amino acid residues.

[0016]

The antigenic outermembrane protein or immunogenic fragment thereof can also be synthesized by solution phase peptide synthesis, solid phase peptide synthesis, or other method employed in the peptide synthesis, and if desired, the synthesis may be accomplished by using an automated peptide synthesizer. The synthesis may be carried out in accordance with the procedures

described in "Lectures on Biochemical Experiments, 1, Chemistry of Proteins IV" edited by The Japanese Biochemical Society, published from Tokyo Kagaku Dojin, 1975; Izumiya et al, "Fundamentals and Experiments of Peptide Synthesis", Maruzen, 1985; "Lectures on Biochemical Experiments, Second Series, 2, Chemistry of Proteins, Second Volume" edited by The Japanese Biochemical Society, published from Tokyo Kagaku Dojin, 1987; and the like.

[0017]

The antigenic outermembrane protein or immunogenic fragment thereof may also be prepared from the DNA or the RNA having corresponding nucleotide sequence by using a genetic engineering technique. (For example, see "Lectures on Biochemical Experiments, Second Series, 1, Methods in Gene Study I" edited by The Japanese Biochemical Society, published from Tokyo Kagaku Dojin, 1986; "Lectures on Biochemical Experiments, Second Series, 1, Methods in Gene Study II" edited by The Japanese Biochemical Society, published from Tokyo Kagaku Dojin, 1986; and "Lectures on Biochemical Experiments, Second Series, 1, Methods in Gene Study III" edited by The Japanese Biochemical Society, published from Tokyo Kagaku Dojin, 1987).

[0018]

When the immunogenic fragment is a low molecular weight substance, the immunogenic fragment may be used as a conjugate with a carrier. Exemplary carriers which may be used include keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), human serum albumin (HSA), avian serum albumin, poly-L-lysine, polyalanyl lysine, dipalmityl lysine, tetanus toxoid, and polysaccharides. The immunogenic fragment may be conjugated with a carrier by a

method known in the art such as glutaraldehyde method, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide method, maleimidobenzoyl-N-hydroxysuccinimide ester method, bis-diazotized benzidine method, and N-succinimidyl-3-(2-pyridyldithio)propionate method. The immunogenic fragment adsorbed on a carrier such as nitrocellulose particles, polyvinyl pyrrolidone, or liposomes may also be used as an immunogen.

[0019]

Immunization may be accomplished by administration of the immunogen (the antigenic outer membrane protein or immunogenic fragment thereof as described above) via subcutaneous injection, intramuscular injection, oral administration, or the like. The amount of immunogen inoculated is preferably in the range of 0.01 to 10 mg on protein or peptide basis. A higher antibody titer is obtained by giving booster immunization at an interval of 2 to 10 weeks after the initial immunization. After 2 weeks from the booster, the antibody that specifically reacts with the avian coccidium antigen will be produced not only in the serum of the chicken but also in the egg. The thus immunized antibody will generally retain its high antibody titer for 4 months. If the antibody titer should decline, booster immunization may be given in a manner similar to the one as described above so that the titer will maintain its high level. Antibody activity of the egg can be evaluated by fluorescent antibody technique or enzyme antibody technique.

[0020]

The immunogen is preferably injected as an admixture with an adjuvant. The adjuvant used may be the one known in the art

such as Freund's complete adjuvant, Freund's incomplete adjuvant, aluminum hydroxide adjuvant, and Bordetella pertussis adjuvant.

[0021]

The antibody in the thus produced egg can be used in the form of a solution of the whole egg or the egg yolk, or in the form of a powder prepared using a spray dryer or the like. The whole egg or the egg yolk may also be degreased for use in the form of a water-soluble protein. The antibody may also be purified or crudely purified before its use.

[0022]

The composition of the present invention is administered to birds, and in particular to chickens. A dosage of the composition is generally in the range of 1 to 10^6 folds in terms of antibody titer per day per one individual, although the amount administered is preferably adjusted depending on age by week, sex, breed, and the like of the bird.

[0023]

When the antibody as described above is administered together with a lactic acid bacterium, the symptom of the body weight loss associated with the chicken coccidiosis is better relieved. The lactic acid bacterium may be generally used at a ratio of $10-10^9$ lactic acid bacteria to 1 g of the antibody. The lactic acid bacterium may be administered either simultaneously with the antibody, or at an appropriate interval from the administration of the antibody.

[0024]

The lactic acid bacterium used for such purpose is not limited to any particular species, and any lactic acid bacterium belonging to Lactobacillus, Bifidobacterium, Streptococcus,

Enterococcus, or Lactococcus may be used either alone or as a mixture of two or more species or strains. Examples of lactic acid bacteria belonging to the genus Lactobacillus include Lactobacillus acidophilus, L. bulgaricus, L. casei, L. fermentum, L. rhamnosus, L. paracasei, L. lactis, L. plantarum, L. reuteri, L. rhamnosus, and L. salivarius; the lactic acid bacteria belonging to genus Bifidobacterium include Bifidobacterium adolescentis, B. bifidum, B. breve, B. infantis, B. longum, B. pseudolongum, and B. thermophilum; the lactic acid bacteria belonging to genus Streptococcus include Streptococcus agalactiae, S. salivarius, and S. thermophilus; the lactic acid bacteria belonging to genus Enterococcus include Enterococcus faecalis and E. faecium; and the lactic acid bacteria belonging to the genus Lactococcus include Lactococcus lactis.

[0025]

When the antibody as described above is administered together with an antibody from an egg of the chicken immunized with Clostridium perfringens (hereinafter referred to as "anti-Clostridium perfringens antibody"), the symptom of the body weight loss associated with the chicken coccidiosis is better relieved. The anti-Clostridium perfringens antibody may be generally used at a ratio of 0.01-100 g to 1 g of the anti-chicken coccidiosis antibody. The anti-Clostridium perfringens antibody may be administered either at the same time as with the anti-chicken coccidiosis antibody, or at an appropriate interval from the administration of the anti-chicken coccidiosis antibody.

[0026]

The anti-Clostridium perfringens antibody may be prepared by a process similar to the process used for preparing the

anti-chicken coccidiosis antibody as described above, except that the immunogen used is the *Clostridium perfringens*. The anti-*Clostridium perfringens* antibody and the anti-chicken coccidiosis antibody can also be produced at the same time by simultaneous immunization with the *Clostridium perfringens* and the antigenic outermembrane protein or immunogenic fragment thereof as described above.

[0027]

Examples of antigens which may be used in producing the anti-*Clostridium perfringens* antibody include the protein described in *J. Anim. Sci.*, 75: 19-25, 1997; the toxin described in *Avian Diseases*, 21: 241-255, 1977; the protein described in *Vet. Rec.*, 120: 435-439, 1987; the protein described in *Vaccine*, 11: 1253-1258, 1993; and the protein described in *FEMS Immunol. Med. Microbiol.*, 7: 321-336, 1993.

[0028]

The anti-chicken coccidiosis composition of the present invention may be fed as a solution containing each effective component at a concentration of 0.001%-10%, or may be fed as powder, granules, tablets or paste which is mixed with a feed so that the each effective component is contained in the feed at a concentration of 0.001%-10%. Also, the anti-chicken coccidiosis antibody may be administered in the enteric coated dosage form to prevent digestion and decomposition of the composition in the stomach.

[0029]

[Examples]

[Example 1]

A 4 week old chicken was infected by orally administering 2×10^6 oocysts of each of *Eimeria acervulina* NA strain (PE0101), *Eimeria tenella* NM strain (PE0102), and *Eimeria maxima* NT strain (PE0103) which are associated with chicken coccidiosis, and autopsy of the chicken was conducted at 4 days after the infection to obtain intestinal tract and its content. Purification of the sporozoite and the merozoite was conducted in accordance with Avian Diseases, 39: 538-547, 1995. Soluble outer membrane protein from the merozoite of *Eimeria acervulina* (NA strain) was subjected to SDS-PAGE to obtain outer membrane protein of 18 to 27 kD (Avian Diseases, 44: 379-389, 2000). A solution containing 0.5 mg/ml of this outer membrane protein was emulsified with Freund's incomplete adjuvant, and the resulting emulsion was injected to 12 week old hen into its left and right pectoralis muscles at a dose of 1 ml for each muscle for initial immunization. 6 weeks after the initial immunization, the hen was given the second immunization in a similar manner. 2 weeks after the second immunization, antibody titer of the anti-chicken coccidiosis antibody in the blood of the hen was measured by enzyme-linked immunosorbent assay (ELISA) (Avian Diseases, 44: 379-389, 2000), and the antibody titer was 30,000 to 120,000 folds. The egg of this hen had an antibody titer of 30,000 to 120,000 folds, and this antibody titer level was maintained for another 4 months. The eggs were gathered, and a whole egg powder comprising an egg antibody was produced by spray drying. The powder produced had an antibody titer of 60,000 folds. When this antibody was evaluated for its cross reactivity with the sporozoite and the merozoite by indirect immunofluorescent staining (Avian Diseases, 44: 379-389, 2000), this antibody specifically stained the surface of the sporozoite and the merozoite of the

Eimeria species (Eimeria acervulina, Eimeria tenella, and Eimeria maxima) confirming that this antibody was a common antibody for these species.

[0030]

[Example 2]

The anti-chicken coccidiosis antibody produced in Example 1 is added to a broiler feed to evaluate effects of the antibody on the chickens that had been experimentally infected with chicken coccidiosis (Eimeria tenella) (Avian Disease, 31: 112-119, 1987). The animal used in this evaluation was "Chunky" which is a strain specifically developed for broiler production, and the antibody was administered by adding the antibody to the standard broiler feed for early fattening period (SDB No.1: Research Institute For Animal Science In Biochemistry & Toxicology) at a concentration of 0.1%, 1%, and 10%. The chickens were infected with oocysts of Eimeria tenella (ET strain) at 1000 oocysts/animal (J. Protozool, 9: 154-161, 1962), and each group includes 10 chickens. The observation period of the chickens after the infection was 2 weeks. The chickens were mainly observed for their weight gain and feed conversion ratio. O.P.G. (oocysts per gram of faeces) and cecal lesion were also observed ("Coccidiosis in Chicken", editorial supervision by K. Tsunoda, published by Chikusan Shuppan, 1983). Significant effects in the weight gain were then confirmed in the group that had been given the feed comprising 0.1% of the antigen, and significant effects in the feed conversion ratio were confirmed in the group that had been given the feed comprising 1.0% of the antigen.

[0031]

As evidence from the results, by administration of the feed comprising 0.1% or more of the antibody to the chickens attacked by the chicken coccidiosis, the conditions of the chickens including the weight gain and the feed conversion ratio are significantly improved. The overall effect of the test substance was determined by using the scores of each observation, and the antibody proved to be very effective.

[0032]

[Table 1]

	Uninfected group	Group infected with <i>E. tenella</i>			
	Group with no addition to the feed	Proportion of the antibody added to the feed			
	0%	0%	0.1%	1%	10%
Average weight gain (g)	731	578	622	620	666
Relative weight gain (%) ^{a)}	100	79	85	85	91
Average feed intake (g)	879	885	910	862	915
Feed conversion rate ^{b)}	1.20	1.53	1.46	1.39	1.37
Survival rate(%)	100	100	100	100	100
OPG (x 10 ⁴) at 7th day	0.0	9.7	5.1	4.2	0.4
Oocyst score ^{c)}	0	40	20	10	5
Cecal lesion score ^{d)}	0	20	10	5	5
ACI index ^{e)}	200	119	155	170	181
			Slightly effective	Considerably effective	Very effective

[0033]

a): Relative weight gain (%) = [weight gain of the infected group] ÷ [weight gain of the uninfected group] x 100

b): Feed conversion ratio = [average feed intake] ÷ [average weight gain]

c): Oocyst score of each group was determined by the calculation and the criteria as described below:

O.P.G. of the medicated group in relation to the control group = [O.P.G. of the medicated group] ÷ [O.P.G. of the infected unmedicated control group) x 100

Criteria: 0: 0 to 1%; +5: 1.1 to 25%; +10: 26 to 50%; +20: 51 to 75%; +40: 76 to 100%.

^{d)}: Cecal lesion score was determined by the following criteria:

0: no lesion observed; +1: a very small number of ecchymosis are scattered along the cecal wall, while the content is normal; +2: small amount of blood is found in the content, and a large number of bleeding lesions are observed; +3: massive hemorrhage or cecal core (cheesy mass of banana-like shape either with blood clots or pale gray color) is found in the cecum, and thickening of cecal wall as well as deformation and atrophy of the cecum are clearly observed; +4: cecum undergoes marked atrophy, and the lesion extends to the rectum. The wall is thickened to the extremity, and blood clotting or cecal core is found in the cecum. Average score was determined for 1 chicken, and the score converted to that of 10 chickens are shown in Table 1.

^{e)}: ACI (Anti-coccidial Index) = [relative percent weight gain + survival rate] - [oocyst score + lesion score]

ACI: 180 or higher: very effective; 160 to 179: considerably effective; 120 to 161: slightly effective; less than 120: not effective.

[0034]

[Example 3]

Effect of the antibody on the chicken which had been experimentally infected with chicken coccidiosis (*Eimeria tenella*) was evaluated by using a feed comprising the anti-chicken coccidiosis antibody produced in Example 1 and a lactic acid

bacterium (*Lactobacillus acidophilus*). The lactic acid bacterium (*Lactobacillus acidophilus*, ATCC 4356 strain) was cultivated in MRS medium, and a powder including 1×10^{11} bacteria/g was produced by lyophilization. The animal used in this evaluation was "Chunky" which is a broiler strain, and the chickens were fed with a standard broiler feed for early fattening period (SDB No.1) having the 0.1% of the antibody and 10^6 /g of lactic acid bacterium added thereto. The chickens were infected with oocysts of *Eimeria tenella* (ET strain) at 1000 oocysts/animal, and each group includes 10 chickens. The observation period of the chickens after the infection was 2 weeks. The chickens were mainly observed for their weight gain and feed conversion ratio. O.P.G. and cecal lesion were also observed. Significant effects were confirmed in the weight gain in the group that had been administered with the antibody and the lactic acid bacterium. Significant effects were also confirmed in the feed conversion ratio in the same group.

[0035]

As evidence from the results, by administration of the feed comprising the anti-chicken coccidiosis antibody and the lactic acid bacterium (*Lactobacillus acidophilus*) to the chickens attacked by the chicken coccidiosis, the conditions of the chickens including the weight gain and the feed conversion ratio are significantly improved. The overall effect of the test substance was determined by using the scores of each observation, and the antibody proved to be very effective.

[0036]

[Table 2]

	Uninfected group	Group infected with <i>E. tenella</i>		
	Group with no addition to the feed	Addition to the feed		
	Control group	Control group	0.1% of antibody	antibody + lactic acid bacterium
Average weight gain (g)	731	578	622	666
Relative weight gain (%)	100	79	85	91
Average feed intake (g)	879	885	910	845
Feed conversion rate	1.20	1.53	1.46	1.27
Survival rate (%)	100	100	100	100
OPG ($\times 10^4$) at 7th day	0.0	9.7	5.1	2.2
Oocyst score	0	40	20	5
Cecal lesion score	0	20	10	5
ACI index	200	119	155	181
			Slightly effective	Very effective

[0037]

[Example 4]

Effect of the feed comprising the anti-chicken coccidiosis antibody produced in Example 1 and the anti-Clostridium perfringens antibody produced from the egg of a chicken immunized with Clostridium perfringens was evaluated on the chicken which had been experimentally infected with chicken coccidiosis (*Eimeria tenella*) and Clostridium perfringens (Avian Disease, 24: 324-333, 1980).

[0038]

Anti-Clostridium perfringens antibody was produced as described below. Clostridium perfringens (ATCC 3624 strain) was cultivated in thioglycolate medium. A solution containing 1 x

10¹⁰/ml of the *Clostridium perfringens* was emulsified with Freund's incomplete adjuvant, and the resulting emulsion was injected to 12 week old hen into its left and right pectoralis muscles at a dose of 1 ml for each muscle for initial immunization. 6 weeks after the initial immunization, the hen was given the second immunization in a similar manner. 2 weeks after the second immunization, antibody titer of the anti-*Clostridium perfringens* antibody in the blood of the hen was measured by cell agglutination test, and the antibody titer was 300 to 1200 folds. The egg of this hen had an antibody titer of 300 to 1200 folds, and this antibody titer level was maintained for another 4 months. The eggs were gathered, and a whole egg powder comprising an egg antibody was produced by spray drying. The powder produced had an antibody titer of 600 folds.

[0039]

The animal used in this evaluation was "Chunky" which is a broiler strain, and the chickens were fed with a standard broiler feed for early fattening period (SDB No.1) having the 0.1% of the anti-chicken coccidiosis antibody and 0.1% of anti-*Clostridium perfringens* antibody added thereto. The chickens were infected with oocysts of *Eimeria tenella* (ET strain) at 1000 oocysts/animal, and infected with *clostridium perfringens* (ATCC 3624 strain) at 10⁷ of the *Clostridium perfringens*/animal, and each group includes 10 chickens. The observation period of the chickens after the infection was 2 weeks. The chickens were mainly observed for their weight gain and feed conversion ratio. O.P.G. and cecal lesion were also observed. Significant effects were confirmed in the weight gain in the group that had been administered with both the anti-chicken coccidiosis antibody and the anti-*Clostridium*

perfringens antibody. Significant effects were also confirmed in the feed conversion ratio in the same group.

[0040]

As evidence from the results, by simultaneous administration of 0.1% of the anti-chicken coccidiosis antibody and 0.1% of the anti-Clostridium perfringens antibody in a feed to the chickens attacked by both the chicken coccidiosis and the Clostridium perfringens, the conditions of the chickens including the weight gain and the feed conversion ratio are significantly improved. The overall effect of the test substance was determined by using the scores of each observation, and these antibodies proved to be very effective.

[0041]

[Table 3]

	Uninfected group	Group infected with <i>E. tenella</i> and <i>Clostridium</i>		
	Group with no addition to the feed	Addition to the feed		
	Control group	Control group	0.1% of anti chicken coccidiosis antibody	0.1% of chicken coccidiosis antibody + 0.1% of anti <i>Clostridium</i> antibody
Average weight gain (g)	731	563	620	629
Relative weight gain (%)	100	77	85	86
Average feed intake (g)	879	880	905	865
Feed conversion rate	1.20	1.56	1.46	1.38
Survival rate(%)	100	100	100	100
OPG ($\times 10^4$) at 7th day	0.0	12.0	6.3	0.1
Oocyst score	0	40	20	0
Cecal lesion score	0	24	12	3
ACI index	200	113	153	183
			Slightly effective	Very effective

[0042]

[Example 5]

The anti-chicken coccidiosis antibody produced in Example 1 is added to a feed to evaluate effects of the antibody on the chickens that had been experimentally infected with chicken coccidiosis (*Eimeria acervulina*) (Avian Disease, 31: 112-119, 1987). The animal used in this evaluation was "Chunky" which is a broiler strain, and the antibody was administered by adding the antibody to the standard broiler feed for early fattening period (SDB No.1) at a concentration of 0.1%, 1%, and 10%. The chickens were infected with oocysts of *Eimeria acervulina* (EA strain) at 1000 oocysts/animal, and each group includes 10 chickens. The

observation period of the chickens after the infection was 2 weeks. The chickens were mainly observed for their weight gain and feed conversion ratio. O.P.G. and small intestine lesion were also observed. Significant effects of the antibody administration was confirmed in the weight gain even in the group that had been given the feed comprising 0.1% of the antibody.

[0043]

As evidence from the results, by administration of the feed comprising the anti-chicken coccidiosis antibody at a rate of 0.1% or more to the chickens attacked by the chicken coccidiosis, the weight gain of the chickens is significantly improved.

[0044]

[Table 4]

	Uninfected group	Group infected with <i>E. acervulina</i>			
	Group with no addition to the feed	Proportion of the antibody added to the feed			
	0%	0%	0.1%	1%	10%
Average weight gain (g)	731	580	630	650	680
Relative weight gain (%)	100	79	86	89	93
Average feed intake (g)	879	826	869	845	820
Feed conversion rate	1.20	1.4	1.38	1.30	1.22
Survival rate(%)	100	100	100	100	100
OPG (x 10 ⁴) at 7th day	0.0	5.0	4.5	4.0	0.3
Oocyst score	0	40	40	40	5
Small intestine lesion score ^{a)}	0	20	5	5	0
ACI index ^{e)}	200	119	141	144	188
			Slightly effective	Slightly effective	Very effective

[0045]

^{a)}: Small intestine lesion score was determined by the following criteria:

0: no lesion observed; +1: white necrotic lesions containing oocysts are scattered in duodenum; +2: an increased number of white necrotic lesions are observed, and the lesion extends to the upper small intestine; +3: an even more increased number of lesions are found, and the lesions are fused with each other. Thickening of the small intestine wall is observed, and the content is watery; +4: duodenum and upper small intestine have become grayish because of the large number of mutually fused lesions. The wall is extremely thickened, and creamy mucus is contained in the interior of the wall. Average score was determined for 1 chicken, and the score converted to that of 10 chickens are shown in Table 4.

[0046]

As shown in Examples 2, 3, and 5, when the anti-chicken coccidiosis antibody or the antibody combined with the lactic acid bacterium was continuously administered to the chicken by mixing them in a feed, symptoms of the chicken experimentally infected with chicken coccidiosis were ameliorated, and the chicken showed an increased weight gain. When the chicken infected with chicken coccidiosis was administered with a mixture of the anti-chicken coccidiosis antibody and the lactic acid bacterium by mixing them in a feed, the conditions of the chicken were remarkably improved including the improvement in the weight gain and the feed conversion ratio. When the anti-Clostridium perfringens antibody was simultaneously administered with the anti-chicken coccidiosis antibody, development of the chicken coccidiosis was prevented, and improvements in the conditions including weight gain and the feed conversion ratio were recognized.

[0047]

[Effects of the Invention]

The anti-chicken coccidiosis composition of the present invention comprising an anti-chicken coccidiosis antibody optionally with a lactic acid bacterium or an anti-Clostridium perfringens antibody specifically reacts with coccidium in chicken. Therefore, the anti-chicken coccidiosis composition of the present invention is very effective in preventing coccidiosis in chicken, and can be used as a substitute for the anticoccidial drugs and vaccines. The composition is also quite effective in ameliorating the symptoms of chicken coccidiosis and in the weight gain.

[Name of Document] ABSTRACT

[Abstract]

[Problems] It is an object to prevent and treat coccidiosis in chicken.

[Means for Solution] Prevention and treatment of coccidiosis in chicken is accomplished by administering an anti-chicken coccidiosis composition adapted for oral administration. This composition contains an antibody obtained from an egg of a chicken immunized with an antigenic outer membrane protein or an immunogenic fragment thereof as an antigen having an immunogenicity common for sporozoite and merozoite of *Eimeria acervulina*, *Eimeria tenella*, and *Eimeria maxima* which are associated with chicken coccidiosis. The composition may optionally contain a lactic acid bacterium and/or an antibody obtained from an egg of a chicken immunized with *Clostridium perfringens*.

[Selected Figure] None